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MONODONTUS RÁRUS, n. sp., novo nematodeo parasito de "MESOMYS GUIARA"

Pelo Dr. LAURO TRAVASSOS.

Vamos descrever um parasito novo de um dos roedores mais raros do Brasil. Só examinámos um exemplar macho; não obstante retardámos a descripção durante muito annos, na esperança de obter outro exemplar do mammifero, o que não foi possivel. Aliás não é de admirar pois trata-se de especie muito rara, conhecida apenas por tres ou quatro exemplares.

Nematodeo de tamanho pequeno com bocca voltada dorsalmente e ampla capsula buccal. Mede de comprimento 4,1 mm; largura 0,38 mm. Cuticula estriada transversalmente; annel nervoso á cerca de 0,28 mm. da extremidade anterior. Bocca conduzindo a uma capsula buccal de 0,088 por 0,080 mm. Esta capsula apresenta ventralmente duas laminas semelhantes ao observado em *Necator* e dorsalmente, no fundo, um dente conico na linha mediana; a entrada do esophago, no fundo da capsula buccal, é guarnecida por quatro saliencias chitinosas agudas e pouco visiveis. O esophago é claviforme, com cerca de 0,26 mm. de comprimento por 0,16 mm. de largura maxima. Bolsa copuladora ampla, funccionando como pinça dorso-ventral, formada pelo lobo dorsal e por um dos lobos lateraes (direito). Tem a seguinte formula de raios bursaes; ventraes com tronco commum; lateral anterior isolado; lateraes medio e posterior com tronco commum (os raios do lobo direito são muito mais fortes que os do lado esquerdo e são implantados um pouco mais atraz); raios dorsaes com tronco commum; raios dorsaes externos nascendo em alturas differentes do dorsal e sendo o direito maior que o esquerdo; dorsal bifurcando-se em ramos de pontas bifidas, o ramo direito mais forte: o comprimento total do raio dorsal é de 0,26 mm. Espiculos delgados e longos tendo uma larga aza lateral na maior parte do comprimento; na extremidade basal tem um forte botão para insersão do musculo retractor e a extremidade distal é fina e curvada em forma de anzol; medem de comprimento cerca de 0,48 mm.; gubernaculo ausente.

Habitat: Intestino delgado de *Mesomys guiara* (BRANTS).

Proveniencia: Angra dos Reis, Estado do Rio.

O roedor foi estudado e determinado pelo nosso prezado amigo Prof. MIRANDA RIBEIRO.

EXPLICAÇÃO DAS FIGURAS.

Fig. 1. Extremidade anterior de face.
« 2. Capsula buccal de face.
« 3. Extremidade caudal.
« 4. Bolsa copuladora.
» 5. Espiculos.

MONODONTUS RARUS n. sp., nouveau nématode parasite du "MESOMYS GUIARA"

Par LAURO TRAVASSOS.

Nous allons décrire un nouveau parasite de l'un des rongeurs les plus rares du Brésil. Nous n'en avons examiné qu'un exemplaire mâle, et avons dû en retarder la description pendant bien des années, dans l'espoir de réussir à nous procurer un autre exemplaire de ce mammifère, ce qui ne nous a pas été possible. Il n'y a pas lieu de s'en étonner, car il s'agit d'une espèce très rare, dont on connaît tout au plus trois ou quatre specimens.

Nématode de petite taille ayant la bouche tournée du côté du dos, et une large capsule buccale. Il mesure en longueur 4,1 mm. et en largeur 0,38 mm. La cuticule est striée transversalement; anneau nerveux à environ 0,28 mm. de l'extrêmité antérieure. Bouche conduisant à une capsule buccale de 0,088 par 0,080 mm. Cette capsule présente, du côté du ventre deux lamelles semblables à celles qui s'observent chez le *Necator* et, dorsalement, dans le fond, une dent conique dans la ligne médiane; l'entrée de l'oesophage, au fond de la capsule buccale, est garnie de quatre protubérances chitineuses, aigües et peu visibles. L'eosophage est claviforme, et mesure environ 0,26 mm. de longueur sur 0,16 mm. largeur maxima. La poche copulatrice est large, et fonctionne comme pince dorso-ventrale, formée par le lobe dorsal et par l'un des lobes latéraux. (droit). Ses rayons boursaux répondent à la formule suivante: les ventraux avec tronc commun; le latéral antérieur isolé; les latéraux moyen et postérieur avec tronc commun (les rayons du lobe droit sont beaucoup plus forts que ceux du côté gauche et sont implantés un peu plus en arrière); rayons dorsaux avec tronc commun; rayons dorsaux externes naissant à des hauteurs différentes du dorsal, celui de droite plus grand que celui de gauche; le dorsal se bifurque en branches aux pointes bifidées, celle de droite plus forte; la longueur totale du rayon dorsal est de 0,26 mm. Spicules minces et longs avec une aile latérale large sur la plus grand; partie de la longueure à l'extrêmité basale se trouve un gros bouton pour l'insertion du muscle rétracteur. L'extrêmité distale est fine et recourbée en forme d'hameçon; ces spicules mesurent environ 0,48 mm. de long; gubernaculum manquant.

Habitat: Intestin grêle du *Mesomys guiara* (BRANTS).

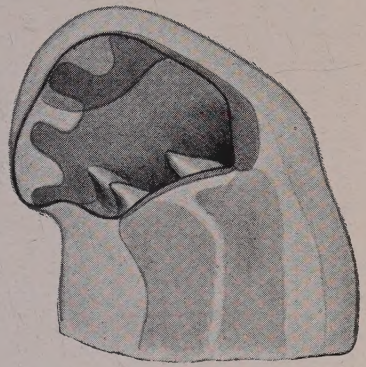
Provenance. Angra dos Reis, Etat de Rio.

Le rongeur a été étudié et détérminé par notre distingué collègue et ami, le Prof. MIRANDA RIBEIRO.

EXPLICATION DES FIGURES:

Fig. 1. Extrêmité antérieure, vue de face.
« 2. Capsule buccale, vue de face.
« 3. Extrêmité caudale.
« 4. Poche copulatrice.
« 5. Spicules.

1



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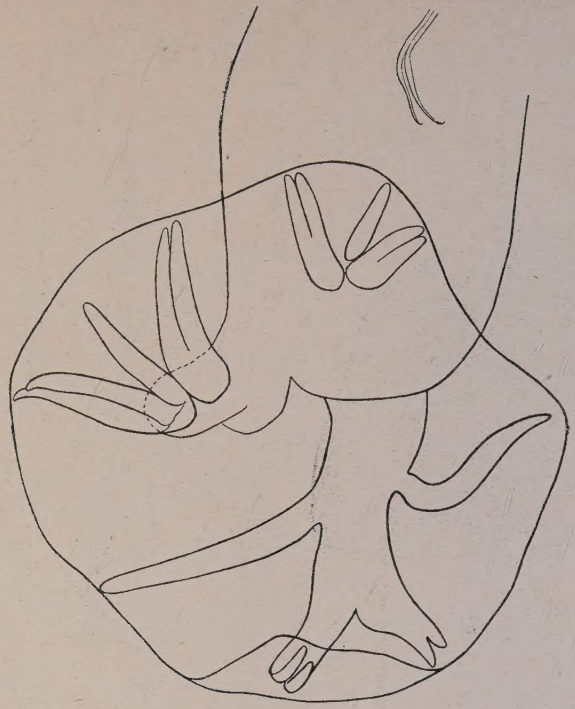

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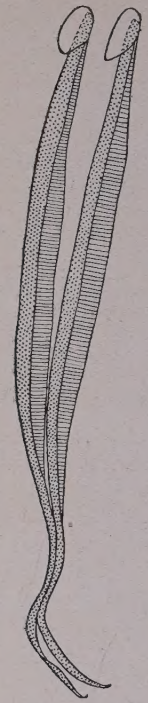

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Molestia de HODGKIN e Corynebacterias

(Nota preliminar)

Pelo Dr. J. G. LACORTE.

O isolamento de bacillos pseudodiphthericos de ganglios de individuos atacados de molestia de HODGKIN data de 1913, quando NEGRI e MIEREMET (1) os descreveram, denominando-os *Corynebacterium granulomatis maligni.* Anteriormente FRAENKEL e MUCH (2) pesquisando o bacillo de KOCH em ganglios nas mesmas condições, conseguiram observar um bacillo gram positivo, não acido-resistente; opinaram por uma forma especial do bacillo de KOCH que no momento procuravam. Em trabalho tambem publicado em 1913, BUNTING e YATES (3) relatam suas primeiras pesquisas sobre o assumpto, tendo isolado o pseudodiphtherico em questão (por elles designado *Corynebacterium hodgkinii*) em 5 casos da referida molestia, sendo de tres delles em cultura pura. Num sexto caso verificaram pelo exame microscopico de esfregaços de ganglios, a presença de microorganismo semelhante, num caso intestinal da molestia. Os mesmos autores e outros isolaram diphtheroides em ganglios lymphaticos de doentes de sarcoma, chloroma, doença de BANTI (no baço) e em associação com a tuberculose. BILLINGS e ROSENOW (4) igualmente os isolaram chegando a tentar vaccinotherapia. Outros autores, principalmente americanos, descreveram a seguir, detalhadamente, diphtheroides isolados de ganglios doentes, porem com diagnostico nem sempre seguro da molestia.

Apresentando-se um caso clinico de molestia de HODGKIN num doente dos Drs. H. MELLO e C. BOMFIM, foi o diagnostico confirmado histopathologicamente pelo Dr. MAGARINOS TORRES, que teve a bondade de nos chamar a attenção para o assumpto, fornecendo-nos as primeiras indicações a respeito do mesmo.

A nosso pedido, os Drs. MELLO e BOMFIM retiraram, por biopsia, com os necessarios cuidados de asepsia, um dos ganglios axillares do referido doente. Depois de reduzido a pequenos fragmentos, foram estes semeados em meios de LOEFFLER e PETROFF. Após 10 dias de estufa a 37°C., observámos, num dos tubos de PETROFF, uma grande colonia ou confluencia de colonias de aspecto humido e com cerla tonalidade rosea. Ao exame microscopico verificámos desde logo tratar-se de um diphtheroide, e as provas seguintes concordaram com o descripto por BUNTING e YATES.

São bastonetes arredondados e curtos, principalmente nas culturas antigas, em que chega a tomar o aspecto de coccos. Nas culturas mais recentes, a sua morphologia é muito variavel: formas alongadas, agrupamentos baccillares, granulações metachromaticas de varios tamanhos, e depois, formas esphericas e de involução.

As culturas iniciaes são abundantes nos meios albuminosos, e as seguintes nos meios communs. Em anaerobiose é fraco o desenvolvimento.

O diphtheroide isolado não foi pathogenico para cobayas, coelhos, ratos e camondongos brancos e saguis. BUNTING e YATES estudaram a acção pathogenica em certos macacos, que não possuimos no momento. Os trabalhos neste sentido não têm sido tentados, ao que nos conste, entre nós, de modo que nos pareceu de certo interesse registrar o presente caso. Por ora é questão grandemente discutida o papel etiologico dos pseudodiphthericos na molestia de HODGKIN.

HODGKIN's disease and Corynebacteriae

(Preliminary note)

By Dr. J. G. LACORTE.

The isolation of pseudodiphtheric bacilli of ganglia of patients suffering from HODGKIN's disease, was made for the first time in 1913, when NEGRI and MIEREMET (1) described them, under the denomination of *Corynebacterium granulomatis maligni*. Former researches carried out by FRAENKEL and MUCH (2) to find KOCH's bacilli in ganglia under the same conditions, resulted in the observation of a gram positive, not acid-fast bacillus; they thought of a special form of the KOCH's bacillus they were looking for at that time. BUNTING and YATES (3) in 1913 report their first researches about the matter; they isolated this pseudodiphtheric bacillus (they named it *Corynebacterium hodgkinii*) in 5 cases of the above disease, three of them in pure culture; they verified the presence of a similar microörganism in a sixth case of an intestinal form of the disease; that was made by means of microscopical examination of ganglia's smears. Those two investigators and others, isolated the diphtheroids in lymphatic ganglia of patients suffering from sarcoma, chloroma, BANTI's disease (in the spleen) and also in association with tuberculosis. They were isolated too by BILLINGS and ROSENOW (4) who even tried the vaccinotherapy. Other investigators, mostly Americans, followed, to describe in detail the isolated diphtheroids of diseased ganglia but not always with a sure diagnosis of the disease.

A clinical case of HODGKIN's disease in one of Dr. H. MELLO's and Dr. C. BOMFIM's patient was available; the diagnosis was histopathologically confirmed by Dr. MAGARINOS TORRES who called our attention to the matter, providing us with the first indications about it.

At our request Dr. H. MELLO and Dr. C. BOMFIM removed by biopsy and with thorough aseptic care, one of the axillary ganglia of that same patient. The ganglion was reduced to small fragments which were smeared in LOEFFLER and PETROFF's media. After ten days incubating at 37°C., we observed in one of the PETROFF's tubes a large colony or confluence of colonies with wet appearance and rose shade. At microscopical examination, we verified immediately we were dealing with a diphtheroid; later proofing agreed with the description of BUNTING and YATES.

These diphtheroids are round and short rods, specially in old cultures, in which they may take the aspect of cocci. In younger cultures, their morphology is most variable: lengthened forms, bacillar groups, me-

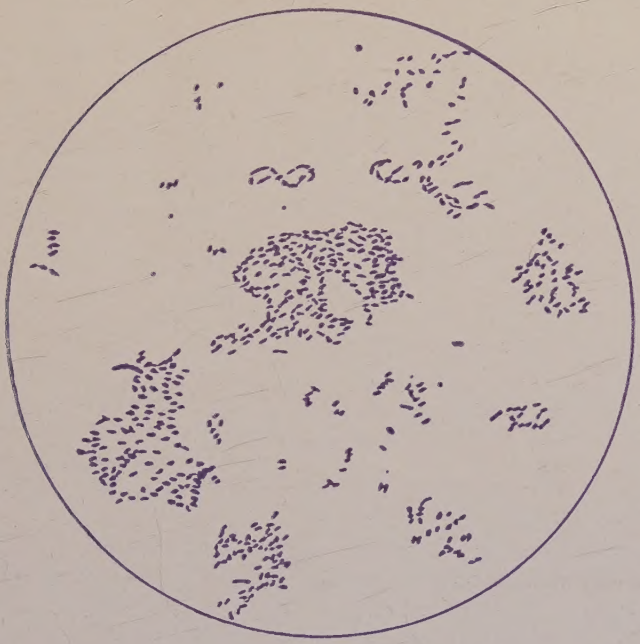

Fig. 1

Fig. 2 A. Leal, del.

Fig. 1—Esfregaço de cultura de 8 dias. Coloração : Methodo de GRAM. Augmento : 1000 d.
Fig. 1—A film of an 8 days' culture. Staining : GRAM's method. Magnified : 1000 d.
Fig. 2—Esfregaço de cultura de 30 horas. Colaração : II. Methodo de NEISSER. Augmento: 1000 d.
Fig. 2—A film of a 30 hours' culture. Staining : II. NEISSER's method. Magnified : 1000 d.

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tachromatic granulations of various sizes, afterwards spherical and involution forms.

The initial cultures ares abundant in albuminous media, and the following ones in common media. In anaerobiotic condition, their delopment is weak. The isolated diphtheroid showed no pathogenic action either to Guinea pigs, rabbits, white rats and mice, and marmosets. BUNTING and YATES studied its pathogenic action in certain monkeys which at present are not available to us. Works in that line seem to us not as having been undertaken in Brazil, so we thought worth to mention the case above. Presently the etiological part played by the pseudodiphtceric bacilli in HODGKIN's disease, remains a greatly discussed question.

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Notas sobre CYCLOCOELIDAE

Pelo Dr. LAURO TRAVASSOS.

Em uma bella publicação sob o titulo acima, na qual WITENBERG, descreve duas novas especies de trematodeos desta familia e faz algumas considerações sobre trabalhos do mesmo assumpto não examinado em sua notavel monographia dos *Cyclocoelidae*.

Uma destas publicações é nossa, e sobre ella faz as seguintes notas:

1) *Ophthalmophagus magalhãesi*. Examinando as duas figuras nossas, admitte tratar-se de duas especies. Não tem razão o brilhante helminthologista, pois uma das figuras representa forma nova, ainda immatura. Observei todas as fórmas intermediarias, pois as differenças notadas não nos passaram despercebidas. Provavelmente devido a publicação ter sido feita em portuguez, não foi bem comprehendida por WITENBERG.

2) *Typhlocoelum neivai*. Julga *T. neivai* dever constituir um novo genero. Estamos inteiramente de accordo com WITENBERG e tinhamos a intenção de fazer o novo genero, que denominamos *Neivaia* n. g., com a seguinte diagnose: *Cyclocelidae*. Intestino sem ceccos; testiculos de fórma alongada sem ramificações; ovario redondo; vitellinos extra-ceccaes, desde a zona do pharynge até abaixo da zona ceccal: alças uterinas intra-ceccaes. Especie typo *N. neivai* (TRAV. 1925).

3) *Typhlocoelum obovale* NEUMANN. Julga *T. obovale* igual a *T. cucumerinum*. Com esta opinião tambem estamos de accôrdo, apenas não tendo examinado o material europeu, não nos julgámos autorizado a fazer a identificação, realizada com toda a autoridade, por WITENBERG.

Rio, 27 – II – 1929.

Exame do sangue na anemia Helminthica

Por GILBERTO G. VILLELA (do Instituto Oswaldo Cruz)

E

J. CASTRO TEIXEIRA (do Hospital Oswaldo Cruz)

Os methodos empregados por SCHMIDT, BUNGE e ABDERHALDEN no estudo da constituição do sangue normal, emprehendidos ha muitos annos, requerem technica pouco pratica e quantidades grandes de sangue, de difficil obtenção quando são necessarias dosagens repetidas, como sóe acontecer nos casos pathologicos. Foi só após a introducção dos micromethodos nas analyses dos humores do organismo, que os estudos biochimicos puderam tomar incremento, abrindo novos horizontes á pathologia.

No que diz respeito ás anemias, ainda são escassos os trabalhos relativos á composição chimica do sangue. Ainda menos numerosos são aquelles que visam sómente as anemias produzidas pela infestação de nemathelminthos (ancylostomideos).

Nesta entidade nosologica tornam-se especialmente interessantes as verificações sobre a composição chimica do sangue e suas variações, devido ás complicações a ella associadas no dominio renal e hepatico.

No presente trabalho procurámos estudar alguns elementos inorganicos, taes como o calcio, o potassio e o chloro, e ainda a cholesterina e as albuminas totaes. Em alguns casos determinámos a reserva alcalina cujo interesse se acha ligado ao funccionamento geral do organismo. Os exames hematologicos, que acompanham as observações, mostram o gráo de anemia e os melhoramentos obtidos pelo tratamento.

Os methodos por nós empregados visaram aliar a execução facil do processo á precisão sufficiente de que necessitam as conclusões clinicas.

O sôro, em todos os casos, foi separado do coagulo lógo após a retração deste, sendo então centrifugado e dahi utilizado para as dosagens. Deste modo, as determinações dos chloretos e do potassio não ficaram prejudicadas, o que não aconteceria si o sôro ficasse muitas horas em contacto com os globulos, como observou FRIDERICIA.

Na dosagem do calcio, seguimos a technica de CLARK e COLLIP, e na do potassio a de KRAMER e TISDALL, ambas de emprego hoje generelizado. Na dosagem do potassio, é conveniente deixar o sôro em contacto com o reactivo nitrico-cobaltico durante 50 minutos afim de, si existir excesso de potassio, precipital-o por completo.

Os valores medios normaes para o calcio já foram objecto de estudo de um de nós, feito anteriormente (1) e correspondem a 10,5 mgrs. em 100 c. c. de sôro, sendo que os limites extremos são de 8,9 a 12 mgrs. Quanto ao potassio, os numeros medios são de 16,5 a 22 mgrs. em 100 c. c., como se pode verificar no quadro n. 1.

(1) VILLELA (G. G.) Calcemia normal no Rio de Janeiro. Sciencia Medica Março 1928.

A cholesterina foi dosada pela technica de BLOOR, modificada por SACKETT e adaptada por CARNEIRO FELIPPE ao emprego do padrão do colorimetro de HELLIGE. E' de facil execução e de precisão satisfactoria para os fins clinicos. Baseia-se na extracção pela mistura alcool-ether (3 : 1) directamente do sôro (0,1 c. c. para 6 c. c. da mistura), durante 30 a 40 minutos, centrifugando e evaporando em banho-maria o liquido que sobrenada. Extrae-se o residuo pelo chloroformio, de modo a obter 5 c. c. e neste se pratica a reacção de LIEBERMANN (2 c. c. de acido acetico anhydrido e 0,1 c. c. de acido sulfurico concentrado) e a coloração verde obtida é comparada com a do prisma padrão no colorimetro de HELLIGE. Por meio deste processo os valores da cholesterina são pouco superiores aos dos outros methodos (BLOOR). A media por nós encontrada se acha entre os numeros 170 a 220 mgrs. em 100 c. c.

Quanto á dosagem do chloreto de sodio, empregamos a technica de WHITHORN e FOLIN. O sôro (1 c. c.) é defecado pela mistura de tungstato de sodio a 10 o/o e acido sulfurico 2/3 N (1 c. c. de cada) em balão onde se completa o volume para 10 c. c. Filtra-se e retiram-se 5 c. c. do filtrado, onde se addicionam 10 c. c. de nitrato de prata N/100 e gottas de acido nitrico concentrado e mais o indicador (Alumen ferrico-ammoniacal). Titula-se então pelo rhodanato de ammonio N/10 com microbureta. Normalmente existem no sôro de 510 a 600 mgrs. de chloreto de sodio em 100 c. c.

As albuminas totaes foram determinadas pelo indice refractometrico (apparelho de ABBE-ZEISS). Este methodo dá bons resultados e é de precisão sufficiente para os fins clinicos, conforme foi demonstrado por numerosos pesquizadores (REISS, ROBERTSON, ROWE, NEUHAUSEN e RIOCH). Empregámos a tabella de REISS para as percentagens de albumina relativas ao indice refractometrico, sendo que a temperatura foi corrigida de accordo com o processo de STRAUSS e CHAGÈS, o qual consiste em addicionar 0,0001 ao indice lido, para cada gráo superior a 17,5°C. Tivemos o cuidado de verificar experimentalmente a exactitude desta correcção.

A reserva alcalina só foi feita em alguns casos, não se afastando dos limites normaes. Empregámos o apparelho de VAN SLYKE, de fabricação allemã, como tambem a technica preconizada pelo autor. Seria de maior interesse si, conjunctamente com a reserva alcalina, pudessemos ter determinado o pH e a tensão do CO^2 alveolar, afim de ter uma ideia mais nitida do equilibrio acido-basico e de sua regulação. Em todo o caso, as variações offerecidas pelos plasmas estudados, não mostraram haver afastamentos pronunciados da media normal, assim como tambem os dados clinicos não faziam suppôr nenhum desvio do equilibrio acido-basico.

A primeira vista, os dados para a reserva alcalina podem parecer baixos, mas como se trata de doentes de pouca idade (11 a 16 annos), os numeros se emquadram perfeitamente nas medias consideradas normaes para creanças (46 a 63 c. c. de CO^2 em 100 c. c. de plasma, SCHLOSS e STETSON).

Abaixo reproduzimos os diversos numeros, considerados pelos autores americanos e europeus, como representando as quantidades normaes dos elementos acima referidos.

Em 100 c. c. de sôro:

Calcio — de 9 a 12 mgrs. (MYERS, CLARK e COLLIP, KRAMER).

Potassio — de 16 a 20 mgrs. (KRAMER e TISDALL, DENIS e HOBSON, HAWK e BERGEIM).

Chloretos — de 570 a 620 mgrs. (DENIS e HOBSON, EUGSTER, BIERNACKI).

Cholesterina — de 170 a 200 mgrs. (MYERS, UNDERHILL, BLOOR).

Albuminas totaes — de 7 a 9 grs. (REISS, HAWK e BERGEIM).

Reserva alcalina — de 55 a 75 c. c. de CO^2 (a 0º e 760 mm.) para 100 c. c. de plasma (VAN SLYKE e CULLEN, MYERS).

Afim de melhor apreciar as variações pathologicas, tivemos a opportunidade de dosar, em alguns sôros normaes os elementos em questão. Os resultados por nós obtidos concordam com os referidoss pelos autores extrangeiros, como se pode apreciar na tabella abaixo.

QUADRO N. 1

Em mgrs. para 100 c. c. de sôro.

No.	Potassio	Chloretos	Cholesterina	Albuminas totaes	Reserva alcalina
1	18.2	585	260	7400	63
2	20.3	620	280	8900	53.2
3	19.4	587	178	9000	70.1
4	22.1	510	190	9100	65.4
5	20.0	580	210	8600	58.4
6	19.3	520	220	8900	57
7	18.3	530	250	7800	62.8
8	16.5	520	210	8400	73.3
9	17.8	587	184	8600	77.1
10	19.8	610	199	8700	62.4

Uma das questões mais estudadas em pathologia é a da pathogenia dos edemas. Os nossos resultados não permittem tirar conclusão alguma definitiva sobre a relação existente entre os electrolytos do sôro e a formação dos edemas. Muitos outros pesquizadores teem procurado evidenciar este ponto obscuro, mas as opiniões ainda divergem muito, não permittindo por emquanto, conclusão positiva a respeito.

Entretanto, parece que o calcio tende a baixar, o potassio a permanecer ou diminuir o seu teôr, ao contrario dos chloretos que quasi sempre augmentam. A baixa do calcio, que se observa em alguns casos, talvez corra por conta da diminuição das albuminas totaes, como o demonstraram SALVESEN e LINDER, CIPRIANI e MOLFESE, BOKAY e outros para o sôro de nephriticos. Comtudo, pode haver hypoalbumose sem alteração do calcio, como se nota no caso IX.

O potassio apresentou em geral numeros baixos, facto este que parece se relacionar, de um lado com o gráo de anemia, e de outro com as

perturbações renaes. KRAMER e TISDALL, MYERS e SHORT, KAULFTHEIL e KISH verificaram variar o potassio em relação directa com a percentagem de hematias, de modo que, ao que parece, nas anemias a diminuição deste metal deve se relacionar unicamente com a baixa globular. O caso XI, apezar de muito anemiado, (980.000 hematias por mmcc.) não apresentou baixa sensivel do potassio, mas sim do calcio, e provavelmente este desequilibrio se acha ligado com a producção de edemas pronunciados de que era portador o enfermo. Neste caso a relação K/Ca é elevada (2.2), pois normalmente é de 1.9, evidenciando desequilibrio na isoionia do sangue. Muitos autores acreditam ser esta relação de maior importancia para o conhecimento do estado renal, do que as variações de um unico elemento isolado. Nos casos por nós estudados, esta relação se mostrou variavel em 5 dentre elles (I, II, IV, X e XIII).

Os chloretos se apresentaram augmentados nos doentes I, III, V, VI, VIII, X e XIV. A cholesterina se mostrou mais elevada no começo da doença, abaixando com o tratamento. As albuminas totaes, em quatro casos, deram numeros bem abaixo da media normal, estando pois de accordo com os dados encontrados em outros typos de anemia (ROWE).

Com o tratamento antihelminthico e o restabelecimento das proporções entre o coagulo e o sôro, observa-se.o augmento das albuminas, como tambem do calcio e do potassio. A cholesterina soffre sómente pequenas variações.

As observações clinicas foram feitas no Hospital Oswaldo Cruz, graças á gentileza do Prof. EURICO VILLELA, director do Hospital, a quem depositamos os nossos sinceros agradecimentos. Ao Prof. CARNEIRO FELIPPE que nos encaminhou nas technicas que serviram para a execução deste trabalho, bem como aos ensinamentos a nós prestados, nos consideramos muito agradecidos.

OBSERVAÇÕES CLINICAS.

I.—J. R. A. Reg. 34 Idade: 13 annos. Côr: branco. Sexo: masculino. As dosagens foram feitas logo após os 2 primeiros vermifugos. Face descorada e mucosas idem. Edema leve. Area cardiaca augmentada. Tensão mx 10 e mm. 4 1/2. Ovos de parasitos nas fezes. WASSERMANN negativo. Hemoglobina: 15 o/o. Hematias: 1.705.000 mm³. Apos o tratamento: 66 o/o hemoglobina. Hemaias 3.632.000 mm³.

II.—O. S. Reg. A. 91. Sexo: Masc. Idade: 11 annos. Brasileiro. Internou-se com dyspnéa facil, cançaço, palpitações, tonteiras. Palidez cerea accentuada, mucosas descoradas, edemas dos membros inferiores e da face. Area cardiaca augmentada, sopro mesosystolico. Tensão minima baixa. R. WASSERMANN positiva. Hematologia—45 o/o de hemoglobina. 2.900.000 glob. vermelhos. Exame de fezes—numerosos ovos de ancylostomos. Raros de Trichocephalos. Após tratamento pelo chenopodio, houve melhoria do estado geral. Ausencia de ovos de ancylostomos nas fezes. Albumina negativa na urina. Hematias—3.280.000. Hemoglobina 75 o/o.

III.—S. P. S. Reg. A. 71. Sexo: feminino. Idade: 17 annos. Parda. Canceira, cephalalgia, palpitações. Já teve edema dos membros inferiores. Pelle palida e mucosas descoradas. Sopro mesosystolico e augmento da area cardiaca. Tensão mx. 11 1/2 mm. 5 1/2 (methodo auscultatorio). Hematias—

2.940.000. Hemoglobina 28 o/o. Presença de ovos de ancylostomideos nas fezes. R. WASSERMANN positivo. Albumina, traços. Tratamento pelo chenopodio—Melhoras geraes. 57 o/o de hemoglobina. Hematias—4.120.000 mm³. Albumina negativa.

IV.—J. I. C. Reg. A. 99. Idade: 32 annos. Sexo: Masculino. Pardo. Já teve malaria. Canceira, desanimo para o trabalho. Edema dos membros inferiores. Falta de appetite. Mucosas descoradas e palpebras edemaciadas. Pelle amarello terrosa. Sopro mesosystolico. Dilatação cardiaca. Tensão 11 e 5 1/2 mm. Albumina: traços. Hemoglobina 16 o/o. Hematias: 1.286.000. Ovos de ancylostomos nas fezes. Tratamento pelo chenopodio—Hemogl. 74 o/o Hematias 3.920.000 mm³. Estado geral bom.

V.—J. T. A. Reg. 179. Idade: 14 annos. Sexo: Masculino. Fraqueza, edema e palidez. Sopro mesosystolico, area cardiaca augmentada. Tensão mx 12 e mm. 5 1/2. Hemoglobina 29 o/o. Hematias 1.760.000. Presença de ovos de ancylostomos nas fezes. R. WASSERMANN: positivo. Tmx: 11 e Tmn: 6. Hemoglobina—70 o/o. Hematias: 3.832.000 mm³.

VI.—C. J. M. Reg. A. 56. Idade: 41 annos. Sexo: Masculino. Canceira, palidez, mucosas descoradas. Teve malaria. Leve edema das palpebras e face. Area cardiaca augmentada. Sopro mesosystolico. Tensão mx 12 e mn. 7. Hemoglobina 19 o/o. Hematias 2.460.000 mm³.

VII.—J. P. A. Reg. 194. Idade: 15 annos. Sexo: feminino. Palidez cerea, mucosas descoradas. Grande edema dos membros inferiores, discreto das faces. R. WASSERMANN negativo. Area cardiaca augmentada. Sopro mesosystolico. Hemoglobina: 21 o/o. Hematias: 2.100.000 mm³. Tratamento com chenopodio. Estado geral bom.—Hemoglobina 75 o/o.—Hematias: 4.250.000 mm³.

VIII.—B. R. Reg. A. 20 . Idade: 47 annos. Sexo: Masculino. Branco Portuguez. Agravou-se o seu estado ha 6 mezes. Cançaço, dyspneico quando trabalha. Desanimo para o trabalho. Já tomou vermifugos. Edema dos membros inferiores. Mucosas descoradas: Lingua pallida. Tensão mx. 13 e mn. 7. Sopro mesosystolico, area cardiaca augmentada. Ovos de ancylostomos nas fezes. R. WASSERMANN: negativo. Hemoglobina 25 o/o. Hematias 1.600.000 mm³.

IX.—L. R. Reg. A. 203. Idade: 11 annos. Branca. Brazileira. Cançaço, desanimo para o trabalho, côr pallida. Pouco desenvolvida para a idade. Mucosas descoradas. Leve edema na face e labios inferiores. Tmx. 11 e Tmn. 6. Area cardiaca augmentada. Sopro mesosystolico. Albumina na urina: traços. Hemoglobina: 32 o/o. Hematias: 2.320.000 mm³. Após o tratamento—Hemoglobina: 60 o/o. Hematias: 3.420.000 mm³.

X.—M. R. Reg. A. 206. Branco. Sexo: Masculino. Idade: 16 annos. Internado em estado grave. Anemia intensa. Pelle cerea e labios descorados. Dilatação cardiaca, sopro mesosystolico. Syndroma dysenteriforme. Nivel mental baixo. Doente desde creança. Hemoglobina: 9 o/o. Hematias 980.000 mm³. Ovos de ancylostomideos nas fezes. R. WASSERMANN: negativo.

XI.—M. R. Reg. A. 266. Sexo: Masculino. Idade: 15 annos. Branco. Desanimo. Face descorada. Leve edema dos membros inferiores. Mucosas descoradas. Tensão mx 11 e mm. 6. R. WASSERMANN: negativo. Hemo-

globina 34 o/o. Hematias 1.920.000 mm³. Tratamento pelo chenopodio.— Hemoglobina: 62 o/o.

XII.—F. A. Reg. 3157. Idade: 12 annos. Branco. Canceira. Desanimo. Face e mucosas descoradas, geophagia. Tensão mx 11 e mn 4 1/2. Area cardiaca augmentada. Sopro mesosystolico. Hemoglobina 40 o/o. Hematias 3.100.000 mm³. Ovos de parasitos nas fezes e traços de albumina.

XIII.—A. M. S. Reg. A. 249. Idade: 15 annos. Sexo: Masculino. Pardo. Brasileiro. Estado adiantado de fraqueza, não podendo trabalhar. cançando-se facilmente. Foi sempre doente, côr pallida. Mucosas descoradas. Labios idem. Teve edema dos membros inferiores. Sopro mesosystolico. Augmento da area cardiaca. Tensão mx. 11 e mn. 6. Ovos de ancylostomos, raros de Trichocephalos. Traços de albumina na urina. Hemoglobina: 18 o/o Hematias: 2.200.000 mm³.

XIV.—S. E. S. Brasileiro. Branco. Idade: 24 annos. Sexo: Masculino. Pallidez accentuadissima, cerea. Mucosas muito descoradas. Edema da face, grande edema dos membros inferiores. Pequena ascite. Dyspnéa. Tensão mx. 11. Tensam mn. 5. Grande augmento da area cardiaca. Figado augmentado. Hemoglobina 15 o/o. Hematias: 1.160.000 mm³.

CONCLUSÕES:

I. O calcio variou pouco nos casos observados.

II. O potassio na maioria dos casos se apresentou com numeros baixos. A relação K/Ca variou de 1,7 a 2,3 sendo de 1,9 a normal.

III. Nos doentes graves, o chloreto de sodio se mostrou elevado (casos: I, III, V, VI, VIII, X e XIV). A cholesterina se encontrou sempre elevada.

IV. As albuminas totaes baixam, de accordo com a gravidade dos casos.

V. Os elementos acima referidos voltam ás percentagens normaes após o tratamento antihelminthico.

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QUADRO N. 2

CASO	NOME	Calcio em mgrs. por 100 c.c.	Potassio em mgrs. por 100 c.c.	Relação K/ca	Chloretos em mgrs. por 100 c.c.	Cholesterina em mgrs. por 100 c.c.	Albuminas totaes em grs. por 1.000 c.c.	Reserva alcalina em c.c. de CO^2 a 0º 760 mm.	Hemoglobina (Gowers-Sahli)	Hematias por mm³
I	J. R.	10.8	19.3	1.7	643	260	75	——	66 o/o	3.632.000
II	O. (a)	8.0	16.3	2.3	480	130	61	——	45 o/o	2.900.000
	O. (d)	10.1	18.1	1.8	450	174	87	——	75 o/o	3.280 000
III	S. P. (a)	8.6	18.0	2.1	620	135	82	——	28 o/o	2.940.000
	S. P. (d)	9.9	— —	——	538	—	89	——	57 o/o	4.120.000
IV	J. J. C. (a)	10.0	17.4	1.7	538	220	80	——	16 o/o	1.286.000
	J. J. C. (d)	10.0	18.1	1.8	520	164	93	——	74 o/o	3.720.000
V	J. T. (a)	10.1	19.8	1.9	586	250	75	——	29 o/o	1.760.000
	J. T. (d)	10.2	20.1	1.9	580	176	89	——	70 o/o	3.832.000
VI	C. J. M.	10.1	22.3	2.1	620	244	75	— —	19 o/o	2.460.000
VII	J. P. (a)	9.0	17.2	1.9	420	240	65	68.3	21 o/o	2.100.000
	J. P. (d)	10.2	18.4	1.8	520	220	82	57.9	—	——
	J. P. (d)	10.4	18.3	1.7	510	200	86	58.3	75 o/o	4.250.000
VIII	B. R.	9.4	18.1	1.9	640	200	70	59.3	15 o/o	1.430.000
IX	L. (a)	10.0	19.3	1.9	604	226	55	65.1	32 o/o	2.320.000
	L. (d)	10.3	18.6	1.8	580	220	80	50.4	68 o/o	3.420.000
X	M. R. (a)	9.7	16.7	1.7	610	198	81	56.3	34 o/o	1.920.000
	M. R. (d)	9.9	18.4	1.8	549	160	83	54.1	62 o/o	———
XI	M. (x)	7.9	17.8	2.2	549	178	59	46.1	9 o/o	980.000
XII	F.	9.5	18.0	1.9	596	200	84	62.0	40 o/o	3.100.000
XIII	A.	10.1	17.7	1.7	580	220	76	61.6	18 o/o	2.200.000
XIV	J. E. S.	8.9	19.3	2.1	653	180	60	70.2	15 o/o	1.160.000

Blood study in hook-worm anemia

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AND

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The methods used many years ago by SCHMIDT, BUNGE and ABDERHALDEN for studying the constitution of normal blood, require uneasy technics and large quantities of blood, not practically obtainable when needed for repeated dosages, as generally happens in pathological cases. Biochemical studies were possible to be developed only after introducing the micro-methods in analysis of fluids of the organism, thus opening new and wider fields to pathology.

With regard to "anemia", few have been the works referring to the chemical composition of blood. Still scarcer are those which treat of anemia consecutive to infection by nemathelminths (ancylostomidae).

Under this nosological point of view, verifying the chemical composition of blood and its variation, appears as of specially great interest, in view of the complications occurring together with same in reference to kidney and liver changes.

The present work is intended to study a few inorganic elements, like calcium, potassium and chlorides, as also the cholesterol and the total proteins. In some cases, we determined the alkali reserve, the interest of which is connected with the general working of the organism. The hematological examinations which accompany our observations show the extent of the anemia and the improvements derived from the treatment.

The methods we used, were intended in view of an easy execution allied to the sufficient accuracy required in clinical conclusions.

The serum, was, in every case, separated from the coagulum, immediately after retraction of the latter, being then centrifugated and used afterwards for dosages. This way of doing enabled us to easily determine chlorides and potassium, which would not have been the case, had the serum remained for hours in contact with the globules, as observed by FRIDERICIA.

Calcium determination was effected according to CLARK and COLLIP's method, that of potassium by KRAMER and TISDALL's, both being nowadays of general use. In dosing potassium, it is advisable to leave the serum in contact with the nitric-cobaltic reactive during some 50 minutes, so as to fully precipitate the excess of potassium, if any.

The normal average values for calcium have been object of a previous study of one of us (1) and are equal to 10,5 mgrs, per 100 c.c. of serum, extreme limits ranging from 8,9 to 12 mgrs. Regarding potassium, the average figures are 16,5 to 22 mgrs. per 100 c. c., result shown in table n. 1.

(1) VILLELA G. G. Calcemia normal no Rio de Janeiro, Sciencia Medica March 1928.

Cholesterol was dosed by BLOOR's method, modified by SACKETT and adapted by CARNEIRO FELIPPE, to the use of the standard figures of HELLIGE's colorimeter. It is easily worked out and shows sufficient accuracy for clinical purposes. It is based on extracting directly the serum by the mixture alcohol-ether (3 : 1) (0,1 c. c. of serum of the mixture), during 30 to 40 minutes, centrifugating and evaporating afterwards on water-bath, the overfloating liquid. The residue is then extracted by chloroform, making it up to 5 c. c. in which the LIEBERMANN's reaction is then effected (2 c. c. of anhydride acetic acide and 0,1 c. c. of concentrated sulphuric acid) the green colour thus obtained being henceforth compared with that of the prism of standards in HELLIGE's colorimeter. By this method, the values for cholesterol are a trifle higher than those of other systems (BLOOR). The average we found range between the figures 170 to 200 mgrs. per 100 c.c.

With regard to dosage of the sodium chloride, we applied to WHITHORN and FOLIN's technics. The serum is defecated by the mixture of sodium tungstate 10 o/o, and sulphuric acid 2/3N (1 c. c. of each) in a flask in which it is made up to 10 c. c. The whole is filtered. 5 c. c. of the clear solution are withdrawn and added 10 c. c. of silver nitrate N/100, drops of concentrated nitric acid, plus the indicator (ferric-ammoniacal alum). The titration is then effected by ammonium rhodanate N/10 with the microburette A normal average for serum is 510 to 600 mgrs. sodium chloride per 100 c. c.

Total proteins have been determined by the refractometric index (apparatus of ABBE-ZEISS). This method ensures good results and is sufficiently accurate for clinical purposes, as has been demonstrated by many authors (REISS, ROBERTSON, ROWE, NEUHAUSEN and RIOCH). We made use of REISS's table for the percentages of albumin referring to the refractometric index, correction for temperature being effected in accordance with STRAUSS's and CHAGES's system, this consisting in adding 0.0001 to the index gathered for each grade above 17,5°C. We took care to submit the accuracy of this correction, to experimental test.

The alkali reserve was only effected in a few cases, the same never going beyond normal limits. We used the VAN SLYKE's apparatus of German make, as also the technics recommended by the author. It would have been of great interest if, together with the alkali reserve, we had been able to ascertain the pH and tension of alveolar CO^2, so as to have got a better idea about the acid-basic equilibrium and its regulation. Anyhow, the variations shown in the plasmas we studied, did not prove high differences beyond or within the normal average. Also clinical data did not give way to foresee any break of the acido-basic equilibrium.

At first sight, the data as to alkali reserve may appear to be low, but the case being of young patients, (11 to 16 years old) the numbers remain in perfect standing with the average considered as normal for children, (46 to 63 c. c. of CO^2 per 100 c. c. of plasma, SCHLOSS and STETSON).

We reproduce hereunder the various figures, considered by the American and European authors, as being the normal quantity of the elements above referred to.

In 100 c. c. of serum :

Calcium — 9 to 12 mgrs. (MYERS, CLARK and COLLIP, KRAMER).
Potassium — 16 to 20 mgrs. (KRAMER and TISDALL, DENIS HOBSON, HAWK and BERGEIM).
Chlorides — 570 to 620 mgrs. (DENIS and HOBSON) EUGSTER, BERNACKI.
Cholesterol — 170 to 200 mgrs. (MYERS, UNDERHILL, BLOOR).
Total proteins — 7 to 9 grs. (REISS, HAWK and BERGEIM).
Alkali reserve — 55 to 75 c. c. of CO^2 (at 0o—760 mm.) per 100 c. c. of plasma (VAN SLYKE and CULLEN, MYERS).

In order to get a better appreciation of the pathological variations, we had an opportunity to dose the elements referred to in a few normal serums. The results we got at, are in accordance with the mentioned foreign authors, as shown in the following table:

TABLE N. 1

In mgrs. per 100 c. c. of serum.

No.	Potassium	Chlorides	Cholesterol	Total Proteins	Alkali Reserve
1	18,2	585	260	7400	63
2	20,3	620	280	8900	53,2
3	19,4	587	178	9000	70,1
4	22,1	510	190	9100	65,4
5	20,0	580	210	8600	58,4
6	19,3	520	220	8900	57
7	18,3	530	250	7800	62,8
8	16,5	520	210	8400	73,3
9	17,8	587	184	8600	77,1
10	19,8	610	199	8700	62,4

A question mostly studied in pathology is that referring to pathogeny of "edema". Our own results do not allow to draw any definite conclusion about the relationship that may exist between the electrolytes of serum and the formation of "edema". Many other searchers have tried to bring to light this still unexplained point, but opinions are too different to enable one deciding positivelly on the subject.

Meanwhile, it seems as if the calcium would show a tendency to lower, the potassium to remain at the same rate, or decrease slightly, whereas chlorides almost steadily increase. The fall of calcium, which is observed in some cases, might perhaps be ascribed to the decrease of total proteins, as demonstrated by SALVESEN and LINDER, CIPRIANI and MOLFESE, BOKAY and others for the serum of nephritic patients. Nevertheless, there may exist hypoalbumose without any change in calcium, as may be seen in case N. IX.

Potassium shows generally low figures, which fact seems to be de-

pendent upon, on one part the severeness of "anemia", and on the other part, the kidney troubles. KRAMER and TISDALL, MYERS and SHORT, KAULFTHEIL and KISH verified that the potassium rate varies according to the percentage of red blood corpuscles, so that, as far as would appear in "anemia", the decrease of this metal should depend exclusively on the globular decrease. In case XI, in spite of severe anemia (980.000 red corpuscles per mmcc.) no serious decrease of potassium was shown. On the other hand, calcium had diminished, which break of equilibrium should probably be accounted for by the appearance of serious "edema" on the patient. In this case, the proportion K/Ca is a high one (2,2), 1,9 being the normal, thus showing a break of equilibrium in the isoionia of the blood. Many authors believe this proportion to have a greater importance for the diagnose of the renal condition than the variations of one single element. Among the cases studied by us, this proportion was variable in 5 (I, II, IV, X and XIII).

Chlorides appeared increased in the patients I, III, VI, VIII, X and V. Cholesterol proved to be higher at the beginning of the illness, decreasing with the treatment. Total proteins proved, in four cases, to be lower than the normal average, thus being concordant with the data gathered in other forms of anemia (ROWE).

In the course of the antihelminthic treatment, being restored the proportion between the coagulum and the serum, an increase of proteins is obvious, the same occurring with calcium and potassium. Cholesterol undergoes only slight changes.

The clinical observations were effected at the Oswaldo Cruz Hospital, thanks to the kindness of Prof. EURICO VILLELA, the director of the said Hospital, to whom we feel most obliged. To Prof. CARNEIRO FELIPPE, who guided us through the technical process we used to bring this study to light, and to those of whose teaching we may avail ourselves, we also tender our best thanks.

SUMMARY OF CLINICAL RECORDS.

I.—J. R. A. Reg. 34. Age: 13 years. White. Masculine. Determinations were made immediately after the two first vermifuges. Face and mucosae pale. Slight edema. Cardiac area increased. Arterial tension: maxima 10 and minima 4 1/2. Hook-worms eggs in faesce. WASSERMANN negative. Red blood corpuscles 1.705.000 mm³. Hemoglobin 15 %. Improved: 66 % hemoglobine.—3.632.000 red corpuscles.

II.—O. S. Reg. A. 91. Sex: Masc. age: 11 years. Brazilian. Interned with slight dyspnea, fatigue, palpitations, dizziness. Serious wax-like paleness, pale mucosae, edema in the inferior limbs and in face. Cardiac area increased. Mesostylic breath. Arterial minimum tension low. R. WASSERMANN positive. Hematologia—45 % hemoglobin; 2.900.000 mm³ red corpuscles. Examination of faesce—numerous eggs of ancylostomae, scarcely of Trichocephalus. After treatment by chenopodium, improvement of the general condition. Absence of eggs of ancylostomae in faesce. Negative albumin in urin. Red blood corpuscles—3.280.000 mm³. Hemoglobin 75 %.

III. S. P. S. Sex: feminine. Reg. A. 71. Age: 17 years. Metis. Fatigue, headache, palpitations. Had already edema in the inferior limbs. Skin pale

and mucosae discoloured. Mesostylic breath and increase of cardiac area. Max. tension 11 1/2 Min. 5 1/2 (auscultatory method). Red corpuscles – 2.940.000 mm³. – Hemoglobin 28 o/o. Presence of eggs of ancylostomidae in fesce. R. WASSERMANN positive. Albumin: traces. Treatment by chenopodium – General improvement. 57 o/o hemoglobin. Red corpuscles – 4.120.000 mm³. Albumin negative.

IV. – J. I. C. Reg. A. 99. Age: 32 years. Sex: masculine. Metis. – Had formerly malaria. Fatigue, despondency for work. Edema in inferior limbs. Lack of appetite. Discoloured mucosae and edemized eye-lids. Skin dull yellow. Mesostylic breath. Cardiac dilatation. Tension: 11 and 1/2. Albumin: traces. Hemoglobin 16 o/o. – Red blood corpuscles – 1.286.000 mm³. Eggs of ancylostomae in fesce. Treatment by chenopodium – Hemoglobin 74 o/o. Red blood corpuscles 3.920.000 mm³. – General state good.

V. – J. T. Reg. A. 179. 14 years old. Sex: Masculine. Weakness, edema and paleness. Mesostylic breath. Increase of the cardiac area. Tension: max. 11 and Mn 5 1/2. Hemoglobin 29 o/o. Red corpuscles 1.760.000 mm³. Presence of eggs of ancylostomae in fesce. R. WASSERMANN positive. Treatment. TMx: 11 and Tmn: 6. Hemoglobin 70 o/o.—Red corpuscles 3.832.000 per mm³.

VI.—C. J. M. Reg. A. 59. – Age: 41 years. Sex: masculine. Weariness. Paleness, discoloured mucosae. Had suffered from malaria. Slight edema of eye-lids and face. Increased cardiac area. Mesostylic breath. Tension: max. 12 and Mn 7. Hemoglobin 19 o/o. – Red corpuscles 2.460.000 mm³.

VII. – J. P. Reg. A. 194. – Age: 15. Sex: feminine. Wax-like paleness, discoloured mucosae. Big edema of inferior limbs. Slight in face. R. WASSERMANN negative. Increased cardiac area. Mesostylic breath. Hemoglobin 21 o/o. Red corpuscles 2.100.000 per mmc. Treatment with chenopodium: General state; good. Hemoglobin 74 o/o.—Red blood corpuscles 4.250.000 per mmc.

VIII. – B. R. Reg. A. 201.—Age: 47 years. Sex. Masculine. White. Portuguese. His state grew worse for the last 6 months. Weariness, dyspnea when at work. Strong dislike for work. Had already vermifuges. Edema in inferior limbs. Discoloured mucosae. Pale tongue. Tension: max. 13 and mn 7. Mesostylic breath. Expanded cardiac area. Eggs of ancylostomae in fesce. R. WASSERMANN negative. Hemoglobin 25 o/o.—Red blood corpuscles 1.600.0000 mm3.

IX.—L. R. Reg. A. 203. – Age: 11. White. Feminine. Brazilian. Weariness. Loathing for work. Paleness. Not developed in proportion to her age. Discoloured mucosae. Slight edema of face and inferior lip. Arterial tension: Max. 11 and Mn 6. Increased cardiac area. Mesostylic breath. Hemoglobin: 32 o/o. Red blood corpuscles 2.320.000 mm3. After treatment: Hemoglobin: 68 o/o. Red corpuscles 3.420.000 mm3. R. WASSERMANN: negative.

X. – M. R. Reg. A. 206.—White. Sex: Masculine. Age. 16 Interned in severe condition. Intense anemia. Wax-like skin and discoloured lips. Cardiac dilatation. Mesostylic stroke. Dysenteriform syndroma. Level of mind low. Ill since childhood. Hemoglobin: 9 o/o. Red blood corpuscles 980.000 mmc. Eggs of ancylostomidae in fesce. R. WASSERMANN negative.

XI.—M. R. Reg. A. 266.—Sex: Masculine.—Age 15.—White. Faintness. Discouloured face. Slight edema of inferior limbs. Discoloured mucosae. Arterial tension: max. 11 and mn. 6. Hemoglobin 34 o/o. Red corpuscles 1;920.000 mm³. Treatment by chenopodium: Hemoglobin: 62 o/o. R. WASSERMANN: negative.

XII.—F. A. Reg. 3157. Age: 12 years. White. Weariness.—Faintness. Face and mucosae pale. Geophagy. Arterial tension: max. and mn 4 1/2. Cardiac area increased. Mesostylic breath. Hemoglobin: 40 o/o.—Red corpuscles 3.100.000 mm3. Hookworm eggs in fesce and traces of albumin. R. WASSERMANN: negative.

XIII. A. M. S. Reg. A. 249.—Age 15.—Sex: Masculine. Metis. Brazilian. Advanced state of weariness. No power of working, getting directly exhausted. Has always been ill. Pale colour. Discoloured mucosae. as also the lips. Had edema of inferior limbs. Mesostylic breath. Cardiac area increased. Arterial tension: max. 11 and mn. 6. Eggs of ancylostomae, scarcely of Trichocephalae. Traces of albumin in urin. Hemoglobin: 18 o/o. Re corpuscles: 2.200.000 mm3. R. WASSERMANN; negative.

XIV.—S. E. S. Brazilian. White. Age 24 years.—Sex: Masculine. Very marked paleness, wax-like.—Strongly discoloured mucosae. Edema in the face. Big edema in inferior limbs. Slight ascitis. Dyspnea. Arterial tension: max. 11. Minimum tension: 5. Large increase of cardiac area. Distended liver Hemoglobin 15 o/o. Red corpuscles: 1.160.000 mm3. R. WASSERMANN: negative.

CONCLUSIONS:

I. Calcium shows only slight variation.

II. Potassium, in most cases, showed low figures. The proportion K/Ca varied from 1.7 to 2,3 being the normal 1,9.

III. In severe cases, sodium chloride showed high amount (cases: I, III, VI, V, VIII, X and XIV). Cholesterol showed steadily high figure.

IV. -Total proteins lower according to the severeness of cases.

V.—The elements above referred to get back to normal percentages; consequently to antihelminthic treatment.

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TABLE N. 2

CASES	NAMES	Calcium in mgr. per 100 c.c.	Potassium in mgr. per 100 c.c.	Proportion K/Ca	Chlorides in mgr. per 100 c.c.	Cholesterol in mgrs. per 100 c.c.	Total proteins in gr. per 1.000 c.c.	Alkali Reserve in c.c. of CO^2 at 0° 760 mm.	Hemoglobin (Gowers-Sahli)	Red blood corpuscles per mm^3
I	J. R.	10,8	19,3	1,7	643	260	75	——	66 o/o	3,632,000
II	O. (a)	8,0	16,3	2,3	480	130	61	——	45 o/o	2,900,000
	O. (d)	10,1	18,1	1,8	450	174	87	——	75 o/o	3,280,000
III	S. P. (a)	8,6	18,0	2,1	620	135	82	——	28 o/o	2,940,000
	S. P. (d)	9,9	——	——	538	—	89	——	57 o/o	4,120,000
IV	J. J. C. (a)	10,0	17,4	1,7	538	220	80	——	16 o/o	1,286,000
	J. J. C. (d)	10,0	18,1	1,8	520	164	93	——	74 o/o	3, 20,000
V	J. T. (a)	10,1	19,8	1,9	586	250	75	——	29 o/o	1,760,000
	J. T. (d)	10,2	20,1	1,9	580	176	89	——	70 o/o	3,832,000
VI	C. J. M.	10,1	22,3	2,1	620	244	75	——	19 o/o	2,460,000
VII	J. P. (a)	9,0	17,2	1,9	420	240	65	68,3	21 o/o	2,100,000
	J. P. (d)	10,2	18,4	1,8	520	220	82	57,9	—	——
	J. P. (d)	10,4	18,3	1,7	510	200	86	58,3	75 o/o	4,250,000
VIII	B. R.	9,4	18,1	1,9	640	200	70	59,3	15 o/o	1,430,000
IX	L. (a)	10,0	19,3	1,9	604	226	55	65,1	32 o/o	2,320,000
	L. (d)	10,3	18,6	1,8	580	220	80	50,4	68 o/o	3,420,000
X	M. R. (a)	9,7	16,7	1,7	610	198	81	56,3	34 o/o	1,920,000
	M. R. (d)	9,9	18,4	1,8	549	160	83	54,1	62 o/o	———
XI	M. (x)	7,9	17,8	2,2	549	178	59	46,1	9 o/o	980,000
XII	F.	9,5	18,0	1,9	596	200	84	62,0	40 o/o	3,100,000
XIII	A.	10,1	17,7	1,7	580	220	76	61,6	18 o/o	2,200,000
XIV	J. E. S.	8,9	19,3	2,1	653	180	60	70,2	15 o/o	1,160,000

Intranuclear inclusions in experimental yellow fever

By C. MAGARINOS TORRES.

Examining (1) liver sections from forty-three *Macacus rhesus* inoculated with yellow fever virus (Brazilian (2) and African (3) strains) I found acidophilic intranuclear inclusions (figs. *f*, *g*, *h*, *i*, *j* and *k*) in thirty-one of them.

They were observed still in two injected *M. cynomolgus*, animals whose receptiveness to yellow fever virus was first demonstrated by ARAGÃO (Brasil-Medico, July 7, 1928).

The inclusions were not evidenced, on the other hand, in the liver from six uninoculated *rhesus* monkeys as well as from nine *rhesus* injected with miscellaneous material, *e. g.*, liver from yellow fever patient, NOGUCHI's medium inoculated with virus (several passages), *Bacillus typhosus* culture, etc..

Plate 1 represents besides acidophilic intranuclear inclusions just mentioned, non specific nuclear changes (figs. *b*, *c*, *d* and *e*) dependent on the necrobiosis of the liver cell and which were observed in injected as well as in uninoculated animals. Special reference to them is made here on account of the presence inside the nucleus of eosin-staining substances.

Such cytological minutiae shall be appreciated in well preserved tissues and well stained sections; the study of frozen sections, except in animals where inclusions appeared very abundant, has given untrusty results and it was found that postmortem changes render specially difficult the recognition of inclusions when scarce.

Fig. *a* represents two normal liver cells from uninoculated *rhesus*. The nuclei measure respectively 6 μ 7 by 6 μ 7 and 7 μ by 6 μ 7, the dimensions being the average ones, as rather larger nuclei are at times to be found in some specimens. Chromatin granules appear larger and more numerous at the vicinity of the nucleoli and of the nuclear membrane. No other eosin-staining substance beyond the excentric true nucleolus appears inside the nucleus of a normal liver cell of the *rhesus*.

The nuclei in figs. *f* and *g* is somewhat enlarged as compared with fig. *a*, measuring respectively 7 μ 6 by 7 μ 9, and 8 μ by 7 μ 6, showing as characteristic change the acidophilic mass which is single, presents a semilunar shape and lies at the centre, being well distinct from the nucleolus and separated from the nuclear membrane by a clear area. In early stages of oxychromatic degeneration (figs. *f*, *g* and *h*) chromatin granules

(1) By courtesy of Drs. H. de BEAUREPAIRE ARAGÃO, A. MARQUES DA CUNHA and J. MUNIZ.

(2) From different sources (yellow fever patients at Rio de Janeiro).

(3) Virus sent from New York to B. ARAGÃO.

somewhat smaller than in normal nucleus occur; they become entirely absent, however, in later stages (figs. *i*, *j* and *k*) when an empty, structureless space surrounds the acidophilic inclusion, the only substance demonstrable inside the nucleus.

Fig. *h* represents a "butterfly-like" intranuclear inclusion, a frequent and characteristic picture in monkeys injected with the Brazilian strains (several sources); therefore I was not able to find such stages in the few animals I examined of the African strain series. With regard to the absence of nucleolus in this figure, the plane of sectioning accounts probably for it.

In figs. *i*, *j* and *k* the nuclei are distinctly enlarged measuring respectively 9 μ 5 by 7 μ, 8 μ by 7 μ 6 and 11 μ 4 by 8 μ 5. Nucleolar substances staining dark violet are to be seen either inside the acidophilic inclusions themselves (figs. *i* and *j*) or in their vicinity (fig. *k*); the basichromatin disappears from the inside of the nucleus, condensing itself upon the nuclear membrane where it forms rod-like structures, sometimes separated by a very thin strand of pinkish material which marks the outline of the nucleus.

Moreover, the inclusions are not homogeneous and hyaline in structure but appear more or less granular (figs. *j* and *k*); the more compact the inclusion is, the more intense is its coloration by eosin.

Of course, all liver cells represented in the Plate show fat vacuoles at the cytoplasm besides oxychromatic degeneration.

Figs. *b*, *c*, *d* and *e* represent early stages in necrobiosis of liver cell. Inside the nucleus stained uniformly and at times a deep blue, besides a violet stained granule (nucleolus?) and eosin-staining material, no chromatin granule is visible (*chromatolysis*). The eosin-staining substances form sometimes a ring around the basophilic granule (figs. *d* and *e*); in figs. *b* and *c* the acidophilic material is somewhat diffuse and indistinct.

As a rule, such nuclei are not enlarged, measuring in average 7 μ 9 by 6 μ 6 and although containing acidophilic masses, are sharply distinguishable from those presenting oxychromatic degeneration by the shape and appearance of the eosin-staining substance as compared with the nuclear inclusions, in addition to the diffuse deep blue staining and chromatolysis of the nucleus.

The yellow fever inclusions are confined strictly to the interior of the nucleus, figs. *f*, *g* and *k* showing that they are distinct from the nucleolus, a fact better evidenced in preparations stained with carbol-fuchsin and counter-stained with LOEFFLER's methylene blue and differentiated in alcohol. The nucleoli may appear intensely red in such preparations, while the yellow fever bodies appear blue or blue-violet.

The material composing the inclusions is readily distinguishable from nuclear chromatin in liver sections submitted to FEULGEN's Nuclealfärbung (4); in them, while the chromatin staining blue is found deposited upon the nuclear membrane, the yellow fever bodies remain unstained.

As is the case with herpes, it seems evident that the material which constitutes the yellow fever "inclusions" may partially at least be compo-

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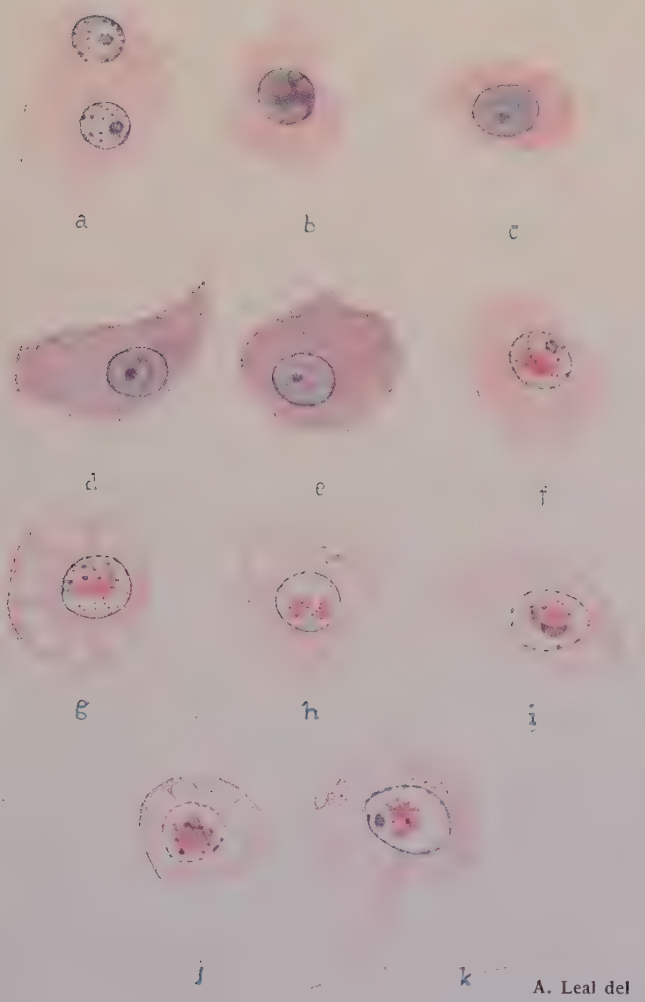
(4) FEULGEN, A. Die Nuclealfärbung in Abderhalden: Handb. d. biol. Arbeitsmeth., Lief. 213, 1926, S. 1055 u. 1071.

C. MAGARINOS TORRES — Intranuclear inclusions in experimental yellow fever (Brazilian strain).



A. Leal del

Liver cells of *M. rhesus*. Helly's fixative; hematoxylin-eosin stain; Zeiss 1/12 im. and oc. comp. 6.

Fig. *a* represent a normal liver cell.

Figs. *b—e* show necrobiotic (non-specific) nuclear changes.

Figs. *f—k* represent characteristic nuclear changes; the inclusions are represented by the pink intranuclear masses.

Fig. *h* represents a "butterfly-like" body.

Figs. *i*, *j* and *k* show the end stages of oxychromatic nuclear degeneration, as well as increase in the size of the nucleus (specially in *k*).

Figs. *f*, *g*, and *i* show acidophilic mass independent of nucleolus.

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EXPLANATION OF PHOTOMICROGRAPHS

Figs. 1, 2, 3, 4, and 5 are photomicrographs, magnification 540 diameters, of liver sections from *rhesus* monkeys. HELLY's fixative; hematoxylin and eosin stain.

Fig. 1 represents normal structure of the nucleus from uninoculated *rhesus*. Some liver cells show fatty degeneration.

Figs. 2 and 3 represent acidophilic intranuclear inclusions at almost every liver cells from *rhesus* injected with yellow fever virus (Brazilian strain). "Entamoeba-like" and "butterfly-like" intranuclear inclusions are a common picture, the "granular stage" being less frequently found. Note in Fig. 2 two typical "butterfly-like" bodies. These figures also show absence of necrotic liver cells.

Figs. 4 and 5 represent acidophilic intranuclear inclusions at liver cells from *rhesus* injected with yellow fever virus (African strain).

Note in fig. 4 hypertrophy of nuclei presenting acidophilic granules and liver cells with "entamoeba-like" intranuclear bodies and in fig. 5 the remarkable amount of basichromatin condensed over the nuclear membrane. Round-shaped necrotic liver cell is evident in fig. 5.

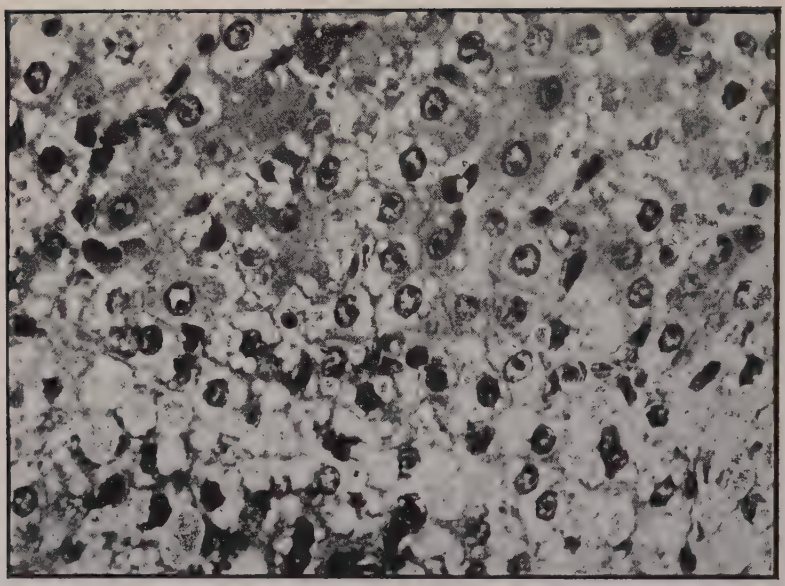

Fig 1

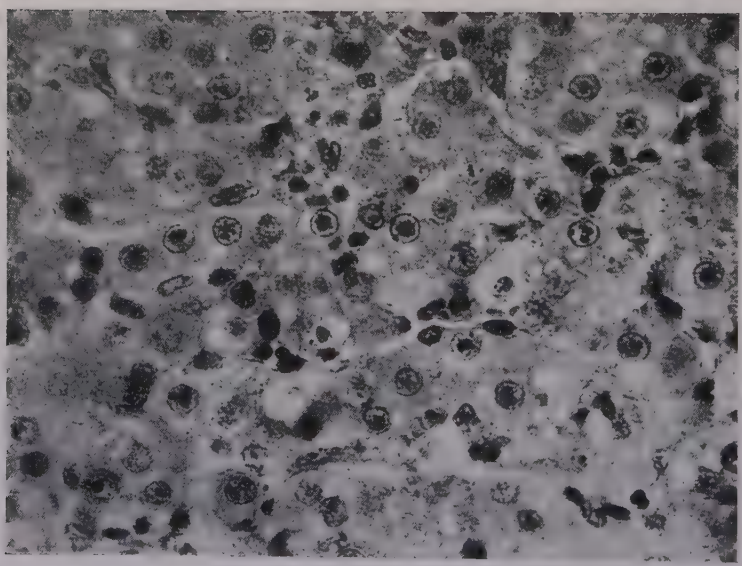

Fig. 2

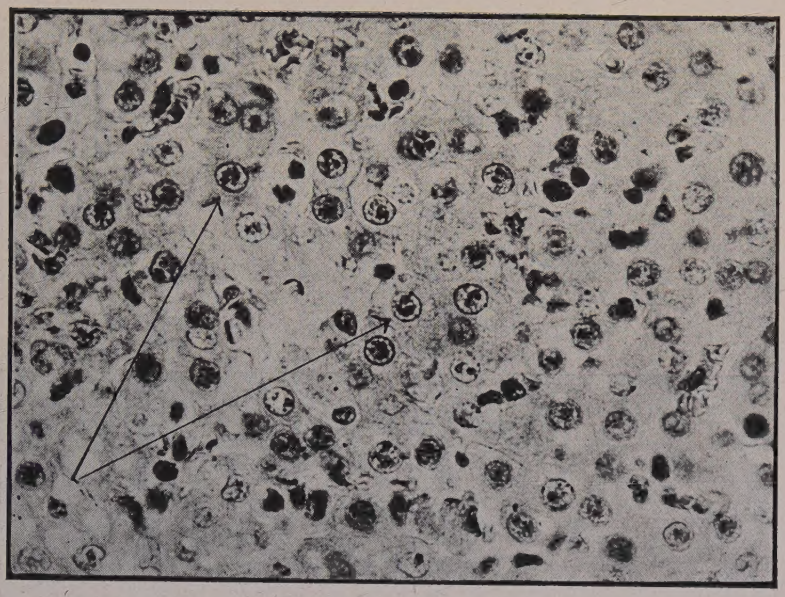

Fig. 3

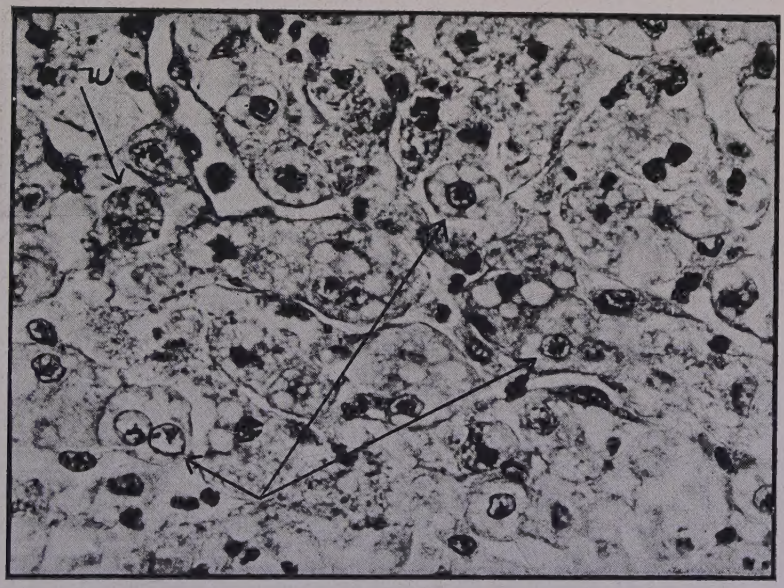

Fig. 4

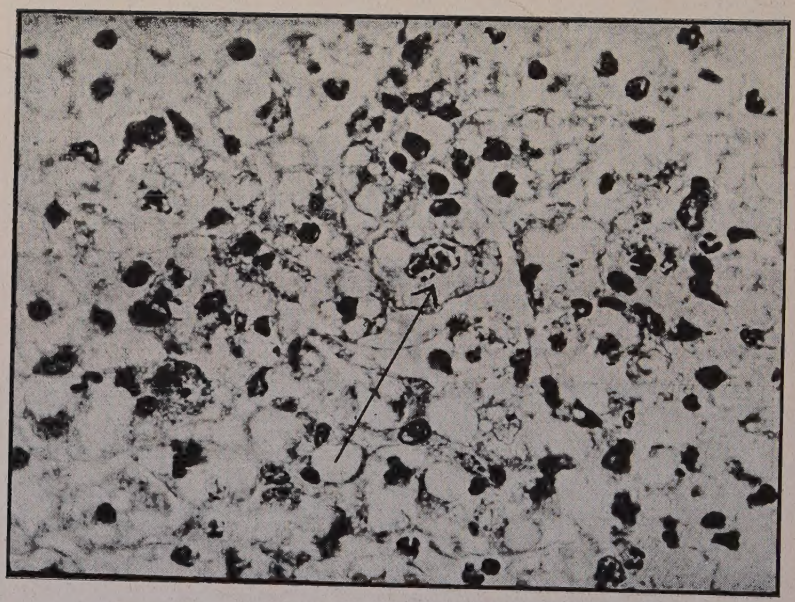

Fig. 5

sed of coagulated nucleoplasm, being surely of a different structure and composition from the two nuclear constituents, chromatin and nucleolus.

It is not definitely known whether the "inclusions" in virus diseases are purely degenerative in nature (*reaction products*) produced by the cell in response to injury or are composed in part at least of the specific virus itself. Nevertheless a desirable work, as emphasizes GOODPASTURE, will be that directed towards "recognizing a constant association of a characteristic type of inclusion with any particular infection, and by identifying it with the lesion so that a diagnosis of the infection on this basis may be possible" (Amer. Jour. of Pathology, 1925, vol. 1, n. 1, pag. 2).

In fact, it was with this object in view that the pathology of experimental yellow fever have been considered in my late papers (5).

Finally, it has appeared that if acidophilic intranuclear inclusions (oxychromatic degeneration) occur both in monkeys infected with the Brazilian and in those with the African strains I was able to examine, discrepancies actually exist between the structure of the inclusion itself or the appearance of recognizable nuclear constituents in both series. They are as follows:

1. In liver cells from monkeys of the African strain series the acidophilic intranuclear material frequently presents itself subdivided in minute masses or granules as reported by STOKES, BAUER and HUDSON (Amer. Jour. of Trop. Med., 1928, vol. VIII, n. 2, pg. 157) and by HUDSON (Amer. Jour. of Pathology, 1928, vol. IV, n. 5, pg. 409); that "granular stage" (evidently an early one) is not so abundant in liver sections from monkeys of the Brazilian strain series, and above all the fragmentation of the acidophilic intranuclear material never is so marked, the granules being there less numerous and generally larger than in the African strain series.

2. In liver cells from monkeys of the Brazilian strain series the "butterfly-like" intranuclear figure (fig. *h*) is a common aspect in early stages of oxychromatic degeneration; such figures are surely not abundant in liver sections from the animals of the African strain series I could examine, and in fact I was unable to demonstrate in such sections "butterfly-like" bodies exactly similar to those recorded in monkeys injected with the Brazilian strain.

3. The amount of basichromatin that becomes marginated forming thick rods over the nuclear membrane at liver cells with intranuclear bodies is apparently more considerable in *rhesus* from the African strain series (predominance of later stages of oxychromatic degeneration?) than in those from the Brazilian strains series (predominance of early stages of oxychromatic degeneration?).

It is believed that differences in the virulence and adaptation of the two strains to *Macacus rhesus* expressed on the other hand by slight necrosis of liver cells at one series (Brazilian strains) and marked necrosis at another (African strain) may account for such discrepancies and surely further data are needed before definite conclusions may be stated as far as differences at nuclear changes in both series are concerned.

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(5) Brasil-Medico, 1928, n. 36, pg. 1001, Sciencia Medica, 1928, n. 9, pg. 427. Mem. Inst. Osw. Cruz, Supl. n. 2, pg. 55, C. R. Soc. Biol, 1928, T. XCIX, pgs. 1655, 1660, 1669 and 1671.